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Usefulness of Gram Staining of Blood Collected from Total Parenteral Nutrition Catheter for Rapid Diagnosis of Catheter-Related Sepsis

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The accuracy of Gram staining of blood drawn from catheters used to administer total parenteral nutrition was compared with paired quantitative blood cultures for the diagnosis of catheter-related sepsis. Gram staining was positive in 11 of 18 episodes of catheter-related sepsis documented by quantitative culture (sensitivity, 61%) but in none of the 5 episodes of fever unrelated to catheter infection. Thus, this procedure enabled the rapid presumptive diagnosis and guidance of antimicrobial therapy for total parenteral nutrition catheter sepsis, with a positive predictive value of 100% and a negative predictive value of 42%.

The diagnosis of catheter-related sepsis is a problem in the management of patients with long-term central venous catheters. Maki et al. (4) showed that the growth of more than 15 CFU from the tip of a catheter is related to catheter colonization or infection rather than contamination. Unfortunately, this technique requires the removal of all possibly infected catheters. When catheters were removed, it has been estimated that 70 to 85% were not infected, and thus were withdrawn unnecessarily (1, 8).

Quantitative cultures of blood collected simultaneously from the catheter and from a peripheral vein allow the diagnosis of catheter-related sepsis while leaving the catheter inserted (2, 3, 5, 6, 9). The catheters that appear to be sterile can thus be left in place. The infected ones can be infused with antibiotics and controlled for sterility after the end of treatment. Only the catheters that remain infected are then removed. Nevertheless, a delay of 24 h is needed for culture results to become available. In our previous experience, quantitative cultures of blood collected from the infected catheters usually grow more than 10^5 CFU/ml (unpublished data). We therefore hypothesized that Gram staining of these samples should be sensitive enough to detect bacterial infection within 1 h of blood collection and to allow for the initiation of an antimicrobial therapy adapted to the Gram staining properties of the microorganisms. In the present study, we assessed the value of Gram staining of blood collected from the catheter as a rapid diagnostic method for catheter-related sepsis in adult patients with long-term total parenteral nutrition (TPN) presenting with fever.

From October 1991 to March 1993, we studied 23 episodes of fever in 21 patients on TPN. The mean age of the patients was 50 years (range, 25 to 77 years). All patients had Hickman-type catheters for a mean period of 4 years (range, 15 days to 8 years) before investigation of a febrile episode. Blood samples (1.5 ml) were simultaneously collected from the TPN catheter and from a peripheral vein into an Isolator 1.5 tube (DuPont Co., Wilmington, Del.) after the first 3 ml of fluid drawn from the catheter had been discarded to flush the catheter with blood. One hundred microliters of blood neat and diluted 10^{-2} collected both from the catheter and from a peripheral vein diluted 10^{-2} was evenly spread onto chocolate agar plates. Colonies were counted after overnight incubation at 37°C in a normal atmosphere. Episodes of sepsis were considered to be catheter related when one of the following criteria was met: (i) blood collected from the catheter grew at least 10 times more of the same organism as blood collected peripherally (definite catheter-related sepsis), and (ii) blood collected from the catheter grew at least 10 CFU/ml of an organism also recovered in standard 10-ml blood cultures (Septi-Chek) collected peripherally within 48 h in the absence of growth from 1.5 ml of blood collected from a peripheral vein in the Isolator 1.5 tube (probable catheter-related sepsis). Gram stains of undiluted blood smears made at the time of inoculation were examined by a trained bacteriologist at a ×1,000 magnification.

Quantitative blood cultures documented the TPN catheter as the definite or probable source of sepsis in 18 of the 23 episodes of fever. In the five other episodes, quantitative blood cultures were negative and the fever was related to another diagnosis. Eleven episodes of catheter-related sepsis were due to staphylococci (10 coagulase-negative staphylococci and 1 *Staphylococcus aureus*) and 7 were due to miscellaneous microorganisms (*Propionibacterium acnes*, 2 episodes; *Lactobacillus* sp., *Candida albicans*, *Pseudomonas fluorescens* and *Enterobacter agglomerans*, *Pseudomonas stutzeri*, and *Enterococcus faecalis*, 1 episode each). Gram staining of blood collected from the TPN catheter was positive in 11 of the 18 episodes of catheter-related sepsis proven by culture (positive predictive value, 100%) and negative in all 5 episodes of fever unrelated to the catheter (negative predictive value, 42%) (Table 1). In the 14 cases of definite catheter-related sepsis, the role of the catheter was proven by positive cultures of the contents of both Isolator 1.5 tubes, with a catheter/periphery CFU ratio ranging from 30 to 10^4. Quantitative cultures of blood collected from the TPN catheter yielded more than 6×10^4 CFU/ml, and Gram staining was positive in 11 of these 14 cases (78%) (9 staphylococci, 1 *Lactobacillus* sp., and 1 *Enterococcus* sp.). For the four episodes of probable catheter-related sepsis, no growth was observed in the culture of 1.5 ml of peripheral blood, and blood from the catheter yielded a mean of 4×10^2

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TABLE 1. Gram staining results for intracatheter blood in 23 febrile episodes among patients with TPN catheters by episode type and blood culture results

Type of episode	No. of episodes	Organism isolated (mean CFU/ml) by quantitative culture of blood collected from:		Mean catheter/periphery CFU ratio	Results of Gram staining of catheter blood
		TPN catheter	Vein		
Definite catheter-related sepsis (n = 14)	8	CNS[a] ($\geq 1 \times 10^5$)	CNS (84)	$\geq 4 \times 10^4$	Gram-positive cocci in clusters
	1	*Staphylococcus aureus* ($\geq 1 \times 10^5$)	*Staphylococcus aureus* (3×10^2)	$\geq 3 \times 10^2$	Gram-positive cocci in clusters
	1	*Enterococcus faecalis* ($\geq 1 \times 10^5$)	*Enterococcus faecalis* (5×10^2)	$\geq 2 \times 10^2$	Gram-positive cocci in chains
	1	*Lactobacillus* sp. ($\geq 1 \times 10^5$)	*Lactobacillus* sp. (20)	$\geq 5 \times 10^3$	Gram-positive bacilli
	2	CNS ($\geq 1 \times 10^5$)	CNS (15)	$\geq 7 \times 10^3$	Negative
	1	*Pseudomonas stuzeri* (6×10^4)	*Pseudomonas stuzeri* (<10)	$\geq 6 \times 10^2$	Negative
Probable catheter-related sepsis (n = 4)	2	*Propionibacterium acnes* (10)	Negative[b]	NP[c]	Negative
	1	*Candida albicans* (270)	Negative	NP	
	1	*Enterobacter agglomerans* (130) *Pseudomonas fluorescens* (10^3)	Negative	NP	
Episode of fever unrelated to catheter infection	5	Negative	Negative	NP	Negative

[a] CNS, coagulase negative staphylococci.
[b] Concomitant qualitative culture of blood (10 ml) positive for the same microorganism as quantitative culture of blood collected from the catheter.
[c] NP, not pertinent.

CFU/ ml (range, 10 to 1,200 CFU/ml) of a microorganism also recovered in concomitant standard blood cultures. In none of these cases did the Gram stains show any microorganisms. Gram stains of blood collected from a peripheral vein were all negative.

The 78% sensitivity and 100% specificity of Gram staining of blood collected from the catheter in our experience of 23 episodes of fever in 21 patients undergoing TPN are comparable to the 87% sensitivity and 94% specificity of the acridine orange leukocyte cytospin test evaluated by Rushforth et al. (7) in 95 episodes of fever in 51 infants receiving intensive care. The sensitivity of Gram staining dropped to 61% if probable catheter-related sepsis episodes (no growth in the culture of 1.5 ml of blood collected from a peripheral vein, but simultaneous isolation from a qualitative blood culture of the same microorganism that grew from blood from the catheter) were considered. Nevertheless, these are not classical criteria for the definition of catheter-related sepsis, and in the four episodes studied, colonization of blood from the catheter was always less massive (4×10^2 CFU/ml versus more than 6×10^4 in episodes of proven catheter-related sepsis). The negative result of Gram staining of blood from the catheter was thus not surprising in these cases.

In conclusion, our findings indicate that in a majority of patients with long-term TPN catheter-related sepsis proven by paired quantitative blood cultures, the presence of a high density of bacteria in the intracatheter blood allowed for the rapid detection of the infecting microorganisms by microscopic examination of Gram-stained blood. In these patients, antimicrobial therapy guided by the Gram staining results could be initiated immediately, without the 24-h delay necessary for culture results to be available. However, a negative Gram stain did not rule out catheter infection. The usefulness of Gram staining should be evaluated for the diagnosis of central catheter-related sepsis in other settings, including in patients receiving long-term chemotherapy or premature neonates under intensive care.

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